Soil Biol. Biochem. Vol. 24, No. 11, pp. 1107–1112, 1992
Printed in Great Britain

0038-0717/92 $5.00 + 0.00
Pergamon Press Ltd

INFLUENCE OF A GENETICALLY MODIFIED ENDOPHYTIC BACTERIUM ON COMPOSITION AND DECOMPOSITION OF CORN RESIDUE

C. F. TESTER
Soil-Microbial Systems Laboratory, ARS, USDA, Beltsville, MD 20705, U.S.A.

(Accepted 20 June 1992)

Summary—I have determined whether the presence of an endophytic bacterium alters the chemical properties of inoculated plants and compared decomposition of colonized residues with control residues in soil. Greenhouse-grown corn (*Zea mays* L.) plants (18 days old) were inoculated with either the endophytic bacteria *Clavibacter xyli* subsp. *cynodontis* (MDE1) or a genetically engineered construction of *C. xyli* subsp. *cynodontis* which was transformed by inserting a gene encoding for production of a δ-endotoxin from *Bacillus thuringiensis* subsp. *kurstaki* (MDR1.3). Leaves and stalks were harvested at maturity and various chemical and physical properties analyzed. In addition, leaf and stalk residues were incorporated into soil to determine the extent of their decomposition in a 63 day laboratory incubation study. Leaf residues from plants inoculated with MDR1.3 retained significantly more water than did those inoculated with buffer or MDE1. Leaves of inoculated plants contained significantly more C than did stalks of inoculated plants. Soluble proteins were the major contributors to the C content of leaves, whereas, soluble carbohydrates were predominant in the stalks. Inoculation with either MDR1.3 or MDE1 appeared to increase the quantity of N present in all plant parts. During early stages, leaf residues contained more readily decomposable substrates than did stalk residues. Leaves from plants inoculated with buffer decomposed significantly more after 63 days than those from plants inoculated with MDE1 or MDR1.3. In contrast, corn stalks inoculated with MDR1.3 decomposed significantly more than those inoculated with buffer or MDE1. Over the 63 day incubation *ca* 33% of the corn residues were decomposed. Although the presence of the endophytes, MDE1 and MDR1.3, during plant growth had some influence on chemical and physical properties of the residues, the extent of residue decomposition in soil (CO_2 evolved in 63 days) was not substantially different.

INTRODUCTION

The bacterium, *Clavibacter xyli* subsp. *cynodontis* (Davis *et al.*, 1984) is commonly found in nature where it colonizes the vascular system of bermudagrass (*Cynodon dactylon* L.) (Liao and Chen, 1981; Davis and Augustin, 1984). This microorganism also colonizes several important crop species including corn (*Zea mays* L.) (Kostka *et al.*, 1988a). A novel delivery system which utilizes naturally occurring plant endophytic bacteria was developed to confer specific insect resistance to plants (Fahey, 1988). *C. xyli* subsp. *cynodontis* has been genetically altered to express the chromasomally inserted δ-endotoxin gene from *Bacillus thuringiensis* subsp. *kurstaki* and antibiotic resistance markers (Turnover *et al.*, 1991). The recombinant strain MDR1.3 is a prototype version of a microbial pesticide that was tested in corn for control of the European corn borer (*Ostrinia nubilalis*) (Kostka *et al.*, 1988b). MDR1.3 systemically colonizes the xylem in stems, leaves and husks of inoculated corn plants and can reach populations in excess of 10^7 CFU g^{-1} plant tissue (Reeser and Kostka, 1988). The endophyte does not survive naturally outside the host plant (Tomasino *et al.*, 1988; Turner and Tomasino, 1988; Davis, 1989), does not persist beyond 18 days in green corn residues incorporated into soil (Turner and Tomasino, 1988) and is not seed transmitted (Reeser and Sommerfeld, 1988).

There are no reports specifically relating physiological interactions of *C. xyli* subsp. *cynodontis* with any annual host plant. Are non-pathological effects of this endophytic bacterium on a non-host plant expressed in altered transformations and partioning of plant biomass components? Moreover, no studies have been conducted to determine whether decomposition of mature, colonized plant residues in soil is altered. The objectives of the present study were to investigate the effect of *C. xyli* subsp. *cynodontis* and the recombinant construction on chemical and physical properties that relate to decomposability of corn residues and to compare decomposition of corn residues grown with or without *C. xyli* subsp. *cynodontis* and the recombinant construction.

MATERIALS AND METHODS

Plant culture and inoculation

Corn (*Zea mays* L. cv. DeKalb T1100) was grown in 4 litre pots containing Terralite Metro-mix 350 (Grace & Co., Cambridge, Mass.) in a greenhouse at Crop Genetics International, Hanover, Md. Nutrients, including trace elements, were supplied twice

weekly by applications of Nutriculture 20-20-20 (Plant Marvel Laboratories, Chicago Heights, Ill.). Iron in the form of Sequesterene 330 Fe (Ciba-Geigy, Greensboro, N.C.) was added at 1.2 g plant^{-1} 2 weeks after planting. Eighteen days after seeding, 10 plants were inoculated with 3 μl of 2.83×10^9 cfu ml^{-1} *C. xyli* subsp. *cynodontis* (MDE1) (designated as "wild-type" hereinafter) suspended in sterile phosphate buffered saline solution (PBS) [10 mM Potassium phosphate (pH 7) and 210 mM NaCl], and another 10 were inoculated with 3 μl of 1.43×10^{10} CFU ml^{-1} *C. xyli* subsp. *cynodontis* strain MDR1.3 (designated as "recombinant" hereinafter) which contained the δ-endotoxin gene from *B. thuringiensis* subsp. *kurstaki* (Fahey, 1988). Ten control plants were inoculated with 3 μl of sterile PBS containing no cells. Both stalk and leaf tissue were inoculated by needle puncture (Vidaver, 1977; Turner *et al.*, 1991). Colonization by both wild-type and recombinant bacteria was confirmed for each plant 15 days after inoculation to ensure that only colonized plants were used. Stem and leaf midrib sections were cut, surface sterilized in 10% sodium hypochlorite solution for 1 min, rinsed with sterile water, dipped into 95% ethanol and flamed. The end of each section was cut back about 5 mm, squeezed and touched onto GCab (selective for wild-type bacteria) and GCanti (selective for both wild-type and recombinant bacteria) media (Anderson and Flaherty, 1988; Turner *et al.*, 1991). Plates containing the imprints were incubated at 27°C for 10 days and then examined microscopically for the presence of endophyte.

The corn was succulent and forming grain at 77 days when 6 plants of each treatment were harvested. Plants were separated into leaves and stalk and dried at 70°C for 9 days before they were ground to <2 mm with a Wiley mill. Oven drying effectively rendered the endophyte non-viable. Ground plant parts were stored at 4°C until analyzed or subsequently used in the laboratory incubation study. The experimental treatments were repeated twice in field studies and twice in the greenhouse. Plant residues from one greenhouse study were used for the specific analyses described in this paper.

Plant residue analyses

For the analyses of soluble carbohydrates and proteins, duplicate 1 g samples of the 6 replicates of each treatment were homogenized in 15 ml of 80% ethanol with a Polytron PT 10/35 homogenizer (Brinkmann Instruments, Westbury, N.Y.) for 30 s. The homogenates were kept at 60°C for 15 min followed by centrifugation at 17,000 *g* for 15 min (Sorvall SS34 rotor). The pellets were re-extracted with 10 ml of 80% ethanol and with 8 ml of 50% ethanol as described above. The supernatants were combined and the volume reduced to 3 ml by flash evaporation (40°C). After adjusting the final volume to 7 ml with deionized water, 60 mg of activated charcoal (6–14 mesh) was added and mixed before incubation for 5 min at 25°C. Charcoal was removed by centrifugation at 10,000 *g* for 10 min and total soluble carbohydrates (SC) were determined by the anthrone reaction (Ashwell, 1957). Individual sugars were determined by gas–liquid chromatography (Li *et al.*, 1988).

Starch and soluble protein were determined by homogenizing the pellets from the ethanol extractions in 10 ml 0.5 N NaOH for 30 s with a Polytron and incubating at 22°C for 15 min before neutralization with 10 ml of 0.5 N acetic acid. The samples were clarified at 10,000 *g* for 10 min, and 1 ml of the supernatant was removed and mixed with 1 ml of amyloglucosidase reagent [1 mg ml^{-1} amyloglucosidase from *Rhizopus* (Sigma Chemical Co., St Louis, Mo) in 0.1 M sodium acetate buffer, pH 4.8]. The mixture was kept for 1 h at 30°C before 30 mg of activated charcoal was added and further incubated at 22°C for 15 min. Charcoal was removed before a 1 ml sample was assayed for glucose. A second sample (2 ml) of the neutralized homogenate was treated with charcoal and 1 ml assayed for glucose. Glucose was determined by the glucose oxidase test (Fleming and Pegler, 1963). Starch glucose was calculated from the difference in glucose content with or without starch digestion by amyloglucosidase. Soluble protein in the neutralized homogenates was assayed by the Bradford method (1976). Non-soluble C components were determined by subtracting the sum of the soluble carbohydrates (42% C) and protein (50% C) from total carbon.

Residue decomposition in soil

The soil used for the decomposition study was an Evesboro loamy sand subsoil (mesic, coated Typic Quartzipsamment, 97% sand) collected from a fescue meadow at the Beltsville Agriculture Research Center. This Upland Coastal plain soil has a pH of 4 and low amounts of total C, N, P and S, and has been used extensively in studies evaluating residue decomposition in soil (Tester *et al.*, 1977; Tester and Parr, 1983; Tester, 1988). The soil was air-dried and sieved ($\leqslant 2$ mm). Lime (3.5 g of CaO: 5 kg soil) was added and mixed thoroughly to yield a pH of 6.4 and stored at 4°C until used in the laboratory incubation study (Tester, 1988). Water content at 33 kPa tension was determined by the pressure-plate method of Klute (1986) for soil, plant residues and 5% (w/w) mixtures of plant residues and soil.

5 g of the appropriate ground plant parts were mixed with 95 g of soil and placed into individual incubation flasks (6 cm dia $\times$ 12 cm high). The mixtures were adjusted to 33 kPa moisture tension, covered with plastic wrap and equilbrated for 3 days at 4°C before initiating the incubation study. Three replicates of each treatment were placed at random on a manifold system controlled at 25°C. The incoming air stream was passed through 4 N NaOH and then through distilled water to maintain the 33 kPa moisture tension (Tester and Parr, 1983). Moisture

tensions of samples were checked at weekly intervals and no corrections were necessary. The effluent gas from each individual sample flask was passed through a tube containing 2 N NaOH for adsorption of evolved CO_2. Empty flasks and flasks containing 95 g of soil were included to determine background concentrations of CO_2 and correct for CO_2 evolved from soil alone.

Residue decomposition was assessed by CO_2 evolution. A specific conductance method was used for CO_2 determinations which utilized a flow-cell connected to a Beckman RA-5 Solu-meter (Beckman Instruments, Cedar Grove, N.J.) with a Fisher (Fisher Scientific, Springfield, N.J.) digital concentration computer and printer (Tester and Parr, 1983). Assay temperature was controlled within ± 0.02°C with a Forma CH/P control system (Forma Scientific, Marietta, Ohio). Coefficients of variation of this analytical technique were <0.2%; thus, extremely small effects of treatment were detectable.

Total C and N of the soil and plant residues were determined by dry combustion using an Erba automated analyzer (Carlo Erba Strumentazione, Milan, Italy). Data were analyzed by ANOVA and GLM (MEANS statement with LSD mean separation options) (SAS Institute, 1987). First-order decomposition rate constants were determined from the integrated rate equation expressed as

$$C = C_0 e^{-kt}$$

where:

$C_0 = C$ concentration at $t = 0$,
$C = C$ concentration at a specific time,
$k =$ first-order decomposition rate constant (day^{-1}),
$t =$ time (days).

RESULTS AND DISCUSSION

Inoculated plants grew and matured at the same rate as did control plants and plant dry mass yields were not significantly different for all three treatments when harvested (Table 1) (no changes in dry mass yields due to inoculation were observed in either the field or greenhouse grown corn plants). All inoculated plants were colonized by $>10^7$ CFU of *C. xyli* subsp. *cynodontis* g^{-1} plant tissue when harvested.

Physical and chemical properties of plant residues

Total C, total N and water retention of corn plant residues are shown in Table 1. Ground leaves from plants inoculated with recombinant cells retained significantly more water than did leaves from either the control or wild-type inoculated plants. Ground stalks from control plants retained significantly more water than those from plants inoculated with either recombinant or wild-type cells. Leaf tissue from all treatments retained significantly more water than did the corresponding stalk tissue, probably the result of differences in chemical composition of the specific plant parts (Table 2). Soluble protein accounted for *ca* 22% of the total C found in leaf tissue, whereas, soluble carbohydrates represented *ca* 20% of the total C of stalk tissue. Collins *et al.* (1990) reported that wheat stems contained more lignin and nonstructural carbohydrates than did wheat leaves, which had higher soluble C. In this study, total C in corn residue probably reflected selective partitioning in plant parts from the different treatments. Even though not significant ($P \leq 0.05$), leaves from wild-type and recombinant inoculated plants contained more C than did leaves from the control plants. In contrast, stalks from both wild-type and recombinant inoculated plants contained somewhat less C (again not significant at 0.05) than did stalks from the control plants. Note that leaves and stalks of control plants contained the same quantity of C. However, the quantity of C found in leaves of inoculated plants was significantly greater than that found in stalks of inoculated plants (Table 1). These total C values correspond to organic matter contents which determine the quantity of water retained by a substance (Klute, 1986). However, the distribution of organic compounds associated with leaves and stalks are different (Table 2) and thus result in differences in water retention.

Starch concentrations of corn residues were <0.5 mg g^{-1} and did not significantly differ among treatments. Stalks contained approximately three

Table 1. Dry weight, water retention, total C and N and C:N ratios of corn residues, and decomposition of corn residues in soil

Material	Property					
	Dry wt	33 kPa†	Total C	Total N	C:N ratio	Decomposition*
	g	g kg^{-1} dry wt				gCO_2-C kg^{-1} residue C
Control leaf	29b‡	2803b‡	482.4ab‡	26.25b‡	18.4	330.0b‡
Wild-type leaf	28b	2799b	505.7a	28.35ab	17.8	322.0c
Recombinant leaf	27b	2903a	502.1a	28.98a	17.3	320.3c
Control stalk	80a	1695c	479.0ab	10.35c	44.9	327.0b
Wild-type stalk	83a	1638d	464.8b	11.35c	41.0	330.9b
Recombinant stalk	84a	1625d	464.7b	11.44c	40.6	338.6a
LSD (0.05)	4	45	23.4	2.20		4.4

*Total decomposition for the entire 63 day incubation.
†Amount of water required to maintain a moisture potential of −33 kPa.
‡Means of six replications followed by the same letter are not significantly different at $P \leq 0.05$.

Table 2. Total carbon, soluble carbohydrates (SC, 42% C), soluble protein (50% C), nonsoluble C components (NSC) and ethanol soluble sugars of corn leaf and stalk samples (percentage of total carbon in parentheses)

	Property							
					Ethanol soluble sugars			
Material	Total C	SC (%)	Protein (%)	NSC (%)†	Fructose	Glucose	Sucrose	Total
	mg g^{-1} dry wt							
Control leaf	482.4ab†	82.4 (7.2)d†	216 (22.4)a*	654 (70.4)	3.2	2.4	33.1	38.7
Wild-type leaf	505.7a	72.5 (6.0)e	224 (22.1)a	699 (71.8)	3.3	2.3	33.2	38.8
Recombinant leaf	502.1a	79.4 (6.6)d	226 (22.5)a	684 (70.9)	3.2	2.9	37.1	43.3
Control stalk	479.0ab	233.4 (20.5)a	97 (10.1)b	692.7 (69)	37.8	35.7	41.7	115.2
Wild-type stalk	464.8b	218.0 (19.7)b	98 (10.5)b	675.4 (70)	35.6	29.5	44.2	109.3
Recombinant stalk	464.7b	208.0 (18.8)c	104 (11.2)b	677.7 (70)	36.4	31.2	45.3	112.9
LSD (0.05)	23.4	6.1	13					

*Means of six replications followed by the same letter are not significantly different at $P \leq 0.05$.
†Nonsoluble C components were determined by subtracting the sum of soluble carbohydrates and protein from total C. Based on leaf and stalk structural components containing *ca* 52 and 48% C respectively.

times the quantity of soluble carbohydrates (SC-anthrone positive) as that detected in leaves (Table 2). The individual sugars (fructose, glucose, and sucrose) totaled *ca* 50% of the SC for each corn residue sample (Table 2). Sucrose accounted for 86% of the sugars found in leaf samples but only 40% of the sugars in stalk samples. The remainder was equally divided between fructose and glucose. No significant differences were detected for the individual sugars (fructose, glucose, and sucrose) found either in the leaves or stalks of individual treatments.

Total N analyses did not reflect the same partitioning trend as seen for total C. Leaves from recombinant inoculated plants contained significantly more N than did leaves from the control plants. Even though not always significant, inoculation with either wild-type or recombinant cells resulted in increased amounts of N in both leaves and stalks as compared to control plants. Stalks contained only 40% of the quantity of N found in leaves. This corresponds to the different types of organic compounds associated with different plant tissues (Table 2). Like total N, soluble protein content of the stalks was 45% of the protein content of leaves.

Residue decomposition in soil

C:N ratios of stalks differed radically from that of leaves, but only minor differences occurred among treatments (Table 1). Total C and N analyses and C:N ratios of plant residues alone are not definitive predictors of subsequent decomposition of residues in soil. The quantity, composition, and C:N ratios of the readily available organic constituents have been found to be more reliable indicators of rate and extent of decomposition of the material (Herman *et al.*, 1977; Reinersten *et al.*, 1984; Jawson and Elliott, 1986). Cumulative CO_2 evolution of the individual treatment residues is shown in Fig. 1 (presented for comparisons of rates of decomposition).

Decomposition rates of leaf substrates from all treatments were indistinguishable during the first 9 days (Table 3), but slight differences were noted

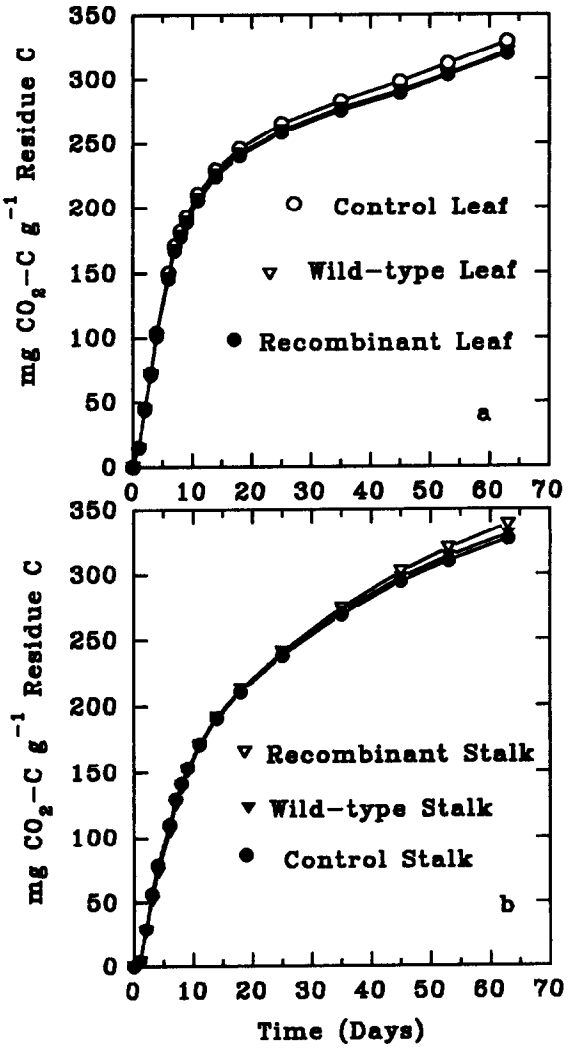

Fig. 1. Cumulative CO_2 evolved during the decomposition of 5 g of either corn leaf or corn stalk residue mixed with 95 g of Evesboro soil. (a) Leaf residue from control and endophyte colonized plants and (b) Stalk residue from control and endophyte colonized plants. See Table 1 for significance. Error bars are smaller than symbols for each data point.

Table 3. Decomposition stages and first-order rate constants† for corn residues mixed uniformly with Evesboro soil

Treatment Material	Stage I (0–9 days)	Stage II (10–35 days)	Stage III (40–63 days)
	rate constants (day^{-1})		
Control leaf	0.02559	0.00386*	0.00258*
Wild-type leaf	0.02515	0.00370	0.00248
Recombinant leaf	0.02494	0.00366	0.00247
Control stalk	0.02022	0.00512	0.00261
Wild-type stalk	0.02038	0.00523	0.00270
Recombinant stalk	0.02000	0.00545	0.00294*

*$P \leq 0.05$. F-test comparisons between treatments. All $r^2 = 0.99$.
†From the integrated rate equation $C = C_0 e^{-kt}$.

thereafter, with substrates in control leaves decomposing significantly more during the 63 days than those from inoculated leaves (Table 1). This either indicated that C-substrates present in inoculated leaves were less available than those present in control leaves or that more C fixation may have occurred. Reinertsen *et al.* (1984) reported that soluble and readily available C were directly related to the initial CO_2 evolved from wheat straw. Herman *et al.* (1977) showed that differences in CO_2 evolution from plant residues during decomposition was proportional to polysaccharide content and inversely proportional to lignin content and C:N ratios.

Decomposition rates of stalk substrates from all treatments were variable (Table 3), resulting in no difference in the quantity of CO_2-C evolved for wild-type inoculated and control stalks. However, they decomposed significantly less than did the recombinant inoculated stalks (Table 1). Stalks from plants inoculated with recombinant contained the lowest C content and decomposed to the greatest extent in this study. The types of organic substances present in stalks from plants inoculated with recombinant may account for the observed differences in decomposition (trend toward more soluble protein but significantly less soluble carbohydrates, Table 2). The small difference in amount of SC present in stalks from plants inoculated with recombinant and the lack of difference in amounts of fructose, glucose and sucrose may indicate that some undetermined factors contributed to the observed differences in decomposition.

On average, corn residues in this study decomposed *ca* 33% during the 63 days incubation (Table 1), which is comparable to the decomposition of wheat straw in a similar incubation study (Tester, 1988). The differences observed in C and N concentrations and rate and extent of decomposition of corn residues in this controlled environment study are so small that they would likely be undetectable in field situations which are subject to wide ranges of environmental fluctations.

Acknowledgements—Sincere appreciation is expressed to Dr John T. Turner of Crop Genetics International, Hanover, Md for supplying the corn residues used in this study, Dr Betty Li, Nutrient Composition Laboratory for the GLC sugar analyses, and Dr Patricia D. Millner, Soil-Microbial Systems Laboratory for critically reviewing the manuscript.

REFERENCES

Anderson J. J. and Flaherty L. (1988) Improved media for the culture of *Clavibacter xyli* subsp. *cynodontis*. *Phytopathology* **78,** 1541.

Ashwell G. (1957) Colorimetric analysis of sugars. In *Methods of Enzymology* (S. Colowick and N. D. Kaplan, Eds), Vol. III, pp. 84–85. Academic Press, New York.

Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72,** 248–254.

Collins H. P., Elliott L. F., Rickman R. W., Bezdicek D. F. and Papendick R. I. (1990) Decomposition and interactions among wheat residue components. *Soil Science Society American Journal* **54,** 780–785.

Davis M. J. (1989) Host colonization and pathogenesis in plant diseases caused by fastidious xylem-inhabiting bacteria. In *Vascular Wilt Diseases of Plants* (E. C. Tjamos and C. Beckman, Eds), pp. 33–50. NATO ASI Series, Vol. H28. Springer, Berlin.

Davis M. J. and Augustin B. J. (1984) Occurrence in Florida of the bacterium that causes bermudagrass stunting disease. *Plant Disease* **68,** 1095–1097.

Davis M. J., Gillaspie Jr A. G., Vidivar A. K. and Harris R. W. (1984) *Clavibacter*: a new genus containing some phytopathogenic coryneform bacteria, including *Clavibacter xyli* subsp. *xyli* sp. nov., subsp. nov. and *Clavibacter xyli* subsp. *cynodontis* subsp. nov., pathogens that cause ratoon stunting disease of sugarcane and bermudagrass stunting disease. *International Journal Systematic Bacteriology* **34,** 107–117.

Fahey J. W. (1988) Endophytic bacteria for the delivery of agrochemicals to plants. In *Biologically Active Natural Products. Potential use in Agriculture, Symposium 380* (H. G. Cutler, Ed.), pp. 120–128. American Chemical Society, Washington.

Fleming I. D. and Pegler H. T. (1963) The determination of glucose in the presence of maltose and isomaltose by a stable, specific enzymic reagent. *Analyst* **88,** 967–968.

Herman W. A., McGill W. B. and Dormaar J. F. (1977) Effects of initial chemical composition on decomposition of roots of three grass species. *Canadian Journal Soil Science* **57,** 205–215.

Jawson M. D. and Elliott L. F. (1986) Carbon and nitrogen transformations during wheat straw and root decomposition. *Soil Biology & Biochemistry* **18,** 15–22.

Klute A. (1986) Water retention: laboratory methods. In *Methods of Soil Analysis, Part 1, Physical and Mineralogical Methods, Second Edition, Agronomy 9* (A. Klute, Ed.), pp. 635–662. American Society of Agronomy, Madison.

Kostka S. J., Reeser P. W., Prunier J. P. and Flynn J. (1988a) *Clavibacter xyli* subsp. *cynodontis*: preliminary distribution studies in the United States and France. *Phytopathology* **78,** 1540.

Kostka S. J., Tomasino S. F., Turner J. T. and Reeser P. W. (1988b) Field release of a transformed strain of *Clavibacter xyli* subsp. *cynodontis* (CXC) containing a delta-endotoxin gene from *Bacillus thuringiensis* subsp. *kurstaki* (BT). *Phytopathology* **78,** 1540.

Li B. W., Marshall M. W., Andrews K. W. and Adams T. T. (1988) Analysis of individual foods for the validation of sugars and starch contents of composited diets. *Journal of Food Composition and Analysis* **1,** 152–158.

Liao C. H. and Chen T. A. (1981) Isolation, culture and pathogenicity to sudangrass of a corynebacterium associated with ratoon stunting of sugarcane and with bermudagrass. *Phytopathology* **71,** 1303–1306.

Reeser P. W. and Kostka S. J. (1988) Population dynamics of *Clavibacter xyli* subsp. *cynodontis* (CXC) and a *CXC/Bacillus thuringiensis* subsp. *Kurstaki* (BT) recombinant in corn (Zea mays). *Phytopathology* **78,** 1540.

Reeser P. W. and Sommerfeld M. L. (1988) Seed transmission of *Clavibacter xyli* subsp. *cynodontis* (CXC) in corn. *Phytopathology* **78,** 1540.

Reinertsen S. A., Elliott L. F., Cochran V. L. and Campbell G. S. (1984) Role of available carbon and nitrogen in determining the rate of wheat straw decomposition. *Soil Biology & Biochemistry* **16,** 459–464.

SAS Institute (1987) *SAS/STAT Guide for Personal Computers*, 6th Edn. SAS Institute, Cary, N.C.

Tester C. F. (1988) Role of soil and residue microorganisms in determining the extent of residue decomposition in soil. *Soil Biology & Biochemistry* **20,** 95–919.

Tester C. F. and Parr J. F. (1983) Decomposition of sewage sludge compost in soil: IV. Effect of indigenous salinity. *Journal of Environmental Quality* **12,** 123–126.

Tester C. F., Sikora L. J., Taylor J. M. and Parr J. F. (1977) Decomposition of sewage sludge compost in soil: I. Carbon and nitrogen transformations. *Journal of Environmental Quality* **6,** 459–463.

Tomasino S. F., Johnson G., Reeser P. W. and Kostka S. J. (1988) Mechanical transmission of a *Clavibacter xyli* subsp. *cynodontis* (CXC) and a *CXC/Bacillus thuringiensis* subsp. *kurstaki* (BT) recombinant. *Phytopathology* **78,** 1541.

Turner J. T. and Tomasino S. F. (1988) Persistance of *Clavibacter xyli* subsp. *cynodontis* (CXC) and a *CXC/ Bacillus thuringiensis* recombinant (CXC/BT) in soil, water, and plant debris. *Phytopathology* **78,** 1541.

Turner J. T., Lampel J. S., Stearmen R. S., Sundin G. W., Gunyuzlu P. and Anderson J. J. (1991) Stability of the δ-endotoxin gene from *Bacillus thuringiensis* subsp. *kurstaki* in a recombinant strain of *Clavibacter xyli* subsp. *cynodontis*. *Applied and Environmental Microbiology* **57,** 3522–3528.

Vidaver A. N. (1977) Maintenance of viability and virulence of *Corynebacterium nebraskense*. *Phytopathology* **67,** 825–827.